Quantifying Variation in Cortical Thickness (Ct.Th) and Volumetric Bone Mineral Density (vBMD) in the Human Radius

Undergraduate Honors Research Thesis

Submitted in partial fulfillment of the requirements for graduation "with Honors Research Distinction in Biomedical Science" in the College of Medicine of The Ohio State University

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Abstract

Computed tomography (CT) is a prevalent clinical instrument providing three-dimensional images to assess bone quality. Quantifying bone quality is important for improving fracture predictive techniques. Cortical thickness (Ct.Th) and volumetric bone mineral density (vBMD) have both been proposed as possible predictors of bone strength across the skeleton; however, data are lacking characterizing the variation within the radius which may impact fracture initiation and propagation patterns. The purpose of this study was twofold: to investigate variation in Ct.Th and vBMD as it related to sex, and to quantify the variation in Ct.Th and vBMD present both along the radial diaphysis and within a cross-section of the radius. Fifty-six *ex-vivo* radii were obtained from 28 male and 28 female age-matched post-mortem human subjects (PMHS) ranging from 60 to 97 years of age (74.9 ± 10.3). A dual x-ray absorptiometry (DXA) scan was performed prior to excision to obtain 33% radius areal bone mineral density (aBMD) values. Radii were scanned using a Philips Ingenuity 64-slice CT resulting in a 0.167mm in-plane resolution. Images were segmented into 30 and 50% volumes of interest (VOI) using SkyScan (Bruker) software. vBMD and Ct.Th measurements were calculated for the total VOI as well as at four independent anatomical regions of interest (ROIs) within each cross-section: anterior, posterior, medial, and lateral. Two-sample t-tests revealed significant sex differences in Ct.Th at both VOI sites, but only for 30% vBMD ($p<0.05$), justifying sex-specific statistical analyses. Linear regression analyses revealed no significant declines in Ct.Th or vBMD with age ($p>0.05$) for males or females in this elderly sample. Paired samples t-tests showed significant differences between total 30% and 50% Ct.Th and vBMD for each sex ($p<0.01$), with Ct.Th being larger at the 50% VOI and vBMD being larger at the 30% VOI for both males and females. No significant differences were found in vBMD between anatomical

ROIs (ANOVA, $p>0.05$); however, significant differences in Ct.Th between ROIs display sex-specific patterns within each VOI ($p<0.05$). Significant differences in vBMD between VOIs were only identified at the medial ROI (paired t-test, $p<0.01$), but significant differences in Ct.Th were found at all ROIs for females, and at all but the lateral ROI for males ($p<0.01$). Lastly, statistically significant positive linear relationships were found for both Ct.Th and vBMD compared to radial aBMD in females (Pearson correlations, $p<0.05$), but only between Ct.Th and radial aBMD in males ($p<0.05$). Overall, significant variation in Ct.Th and smaller amounts of variation in vBMD were found both within the radial cross-section at each ROI and along the radial diaphysis at different VOI sites. Considering the commonality of forearm fractures in elderly individuals, these data can ultimately be utilized to improve the accuracy of injury prediction.

Acknowledgements

I would first like to thank my advisor, Dr. Randee Hunter, for providing me with this invaluable research opportunity and educational experience. Her guidance and support throughout this project have made me a better researcher and have prepared me for my next steps following graduation. I would also like to thank Dr. Karen Briley for her major Ct.Th and vBMD analyses and her help in teaching me how to utilize SkyScan software. I would like to thank Angela Harden for her help with any and all of my questions both in the process of conducting and writing my thesis. I want to thank Zac Haverfield for his help in presenting my research. I also want to thank Akshara Sreedhar for all of her help in the lab and for being such a great friend. I want to thank Dr. Amanda Agnew and the rest of the Injury Biomechanics Research Center for their guidance and support throughout my research. I would also like to thank the College of Medicine, specifically the Biomedical Science major, for the support and guidance during my research career. I would like to thank The Ohio State University Body Donor Program and the donors for their generous gifts. Lastly, I would like to thank my friends and family for their unconditional love and support.

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Introduction

Osteoporosis, a disease traditionally characterized by low bone mass leading to increased fracture risk, is a significant health concern affecting nearly 10 million Americans (Kling et al., 2014). In the United States alone, mortality and morbidity related to osteoporosis accounted for 432,000 hospital admissions and cost $17 billion in 2005 (Kling et al., 2014). The cost of fractures averaged nearly $13,000 per patient in 2016, mostly attributable to room and board, supplies, and the operating room (Weycker et al., 2016). It is estimated that by 2025, annual fracture incidence will rise by 50%, with more than an 87% increase for individuals 65 to 74 years of age resulting in an increase of medical costs (Kling et al., 2014). Due to the growing geriatric population, there will likely be a subsequent increase in hospitalization and treatment costs resulting from fragility fractures. Thus, fracture risk assessment and prevention will become even more crucial in upcoming years.

Osteoporosis is a systemic skeletal disease resulting in low bone mass and structural deterioration coupled with an increase in bone fragility and fracture risk ("Consensus Development Conference: Diagnosis, Prophylaxis, and Treatment of Osteoporosis," 1993) . Due to increased bone loss associated with declining estrogen levels in postmenopausal women, and age-related bone loss in both men and women, the prevalence of osteoporosis increases significantly with age (Centre, 2012). More specifically, the risk of osteoporosis increases from 2% at age 50 to over 25% at age 80 in women (Centre, 2012). As people continue to live longer, the incidence of both osteoporosis and fragility fractures will also increase.

Osteoporosis is responsible for over 9 million fractures annually worldwide, with over 300,000 of those fractures being classified as fragility fractures (Centre, 2012). Fragility fractures are fractures caused by forces not ordinarily capable of causing fracture, also referred to

as low-level trauma (Centre, 2012). Fragility fractures most commonly occur in the vertebrae, proximal femur, and distal radius (Centre, 2012). Osteoporotic fragility fractures can cause both significant pain and disability, leading to a reduced quality of life and possibly reduced life expectancy (Centre, 2012).

In the realm of osteoporotic fragility fractures, distal forearm fractures in the metaphyseal region are among the most common for postmenopausal women (Jerrhag et al., 2017). Distal forearm fractures pose the most threat to the elderly, and such fractures can be severe and have consequential loss of function (Jerrhag et al., 2017). The higher incidence rate of distal forearm fractures in elderly individuals also results in substantial suffering and health care costs. Considering the commonality of forearm fractures in women near or over the age of 55 (Cuddihy et al., 1999)., it is of interest to investigate variation in the radius in order to improve the accuracy of injury prediction.

Additionally, while osteoporosis is common, it is clinically silent until bone quality is significantly low enough to result in injury. Thus, prevention and screening techniques are essential for diagnosis and prevention of injury (Kling et al., 2014). Dual x-ray absorptiometry (DXA) is the current clinical standard for measuring areal bone mineral density (aBMD) and diagnosing osteoporosis ("Who Scientific Group on the Assessment of Osteoporosis At Primary Health Care Level," 2004). While DXA has been validated for its ability to assess aBMD, precision error is inherent in aBMD measurement because it is dependent on the skill of the technologist in charge of positioning the patient (Lewiecki & Lane, 2008). DXA also assigns T-scores based on the comparison of aBMD values to a reference population, resulting in the placement of individuals into broad categories: "normal" (T-score>-1.0), "osteopenic" (-2.5<T-score<-1.0), and "osteoporotic" (T-score<-2.5). However, fracture risk has been shown to

increase independent of T-score changes (Bolotin, 2007). Therefore, the classifications in which DXA places individuals are unreliable and not representative of variation in the population's skeletal health status (Sornay-Rendu et al., 2007).

Quantifying bone quality across various skeletal elements is important in understanding fracture risk, namely for the purpose of improving fracture predictive techniques (Donnelly, 2011; Krug et al., 2010). In comparison to the clinical standard of DXA, a computed tomography (CT) scanner is a prevalent clinical instrument providing images that can be used to assess multiple aspects of bone quality (Donnelly, 2011). Many properties have been proposed as possible predictors of bone strength, with cortical thickness (Ct.Th) and volumetric bone mineral density (vBMD) being two of the most notable predictors of bone strength both across the skeleton and within individual bone (Bonel et al., 2004; Dalzell et al., 2009). Variation in these parameters within the radius has been investigated in previous studies; however, data are lacking in the variation present within a cross-section of the radial cortex (Bonel et al., 2004; Dalzell et al., 2009; Hunter et al., 2017; Long et al., 2015; Yang et al., 2014). Ultimately, due to the increasing prevalence of osteoporotic individuals, especially elderly individuals over the age of 60, developing forearm fractures, it is of significance to quantify the variation present both along the radial diaphysis and within a radial cross-section in order improve fracture risk assessment methods. By improving fracture prediction techniques, the burden of disease, both financially and biologically, may be decreased if fracture risk can be assessed prior to injury. This preventative care mentality has the potential to save patients much of the cost associated with fracture. Additionally, while we know there is variation in bone response to loading, we do not yet fully understand the multi-level contributions to it (Sugiyama et al., 2012). Thus,

characterizing differences in properties within a single long bone is crucial to understanding human variation in bone quality.

The objective of this study was to investigate variation in cortical thickness (Ct.Th) and volumetric bone mineral density (vBMD) as it related to sex and age, and to quantify the variation in Ct.Th and vBMD present both along the radial diaphysis and within a cross-section of the radius.

It was originally hypothesized that there would not be significant declines in either Ct.Th or vBMD with age due to focusing on an elderly population. Additionally, it was hypothesized that there would be significant differences in Ct.Th between four anatomical locations within the cross-section: anterior, posterior, medial, and lateral, but there would not be significant differences in vBMD between anatomical locations. It was also hypothesized that there would be a significant difference in total Ct.Th and total vBMD between sites of interest and between anatomically-specific Ct.Th and anatomically-specific vBMD between sites of interest. Lastly, it was hypothesized that Ct.Th and vBMD would have significant relationships with aBMD.

Methods

Sample Collection

Fifty-six left *ex-vivo* radii were excised from 28 male and 28 female elderly post-mortem human subjects (PMHS) ranging from 60 to 97 years of age (74.9±10.3). PMHS were obtained through The Ohio State University Whole Body Donor Program, and the subjects were age-matched within six years in order to control for morphometric differences with age. Once collected, the *ex-vivo* radii were scanned on a Philips Vereos digital PET/CT with iDose reconstruction software at The Wright Center for Innovation in Biomedical Imaging (WCIBMI)

at The Ohio State University. A resolution of 0.167mm per scan and a slice thickness of 0.671mm were achieved by maintaining consistent acquisition parameters. A QRM cortical phantom with standards of known densities (0-800 mg/cm^3) was included in each scan.

Dual X-ray Absorptiometry (DXA) Scanning

Prior to excision of radii from each PMHS, a whole-body dual x-ray absorptiometry (DXA) scan was performed for a subset of subjects (16 males, 26 females) in order to obtain 33% radius aBMD values. Standard clinical protocol was followed, and raw aBMD values were utilized in this study instead of T-scores.

Segmentation

Whole radii CT scans were imported into SkyScan (Bruker) software for segmentation. A region of interest (ROI) was defined to encompass the entire left radius and saline syringe, and the cortical phantoms were isolated. Dataviewer, a division of SkyScan software, was used to reorient the radius relative to the medullary cavity. The bottom and top slices of the CT scans were identified, with the bottom slice corresponding to the distal articular surface of the radius and the top slice corresponding to the proximal articular surface. Then the number of slices between top and bottom were recorded and used to calculate the total length of the radius in order to obtain the slice number associated with two specific sites of interest (30% and 50% of the total length relative to the distal articular surface) (Figure 1). Ct.Th and vBMD measurements for each volume of interest (VOI) included 10 slices centered around the 30% and 50% locations.

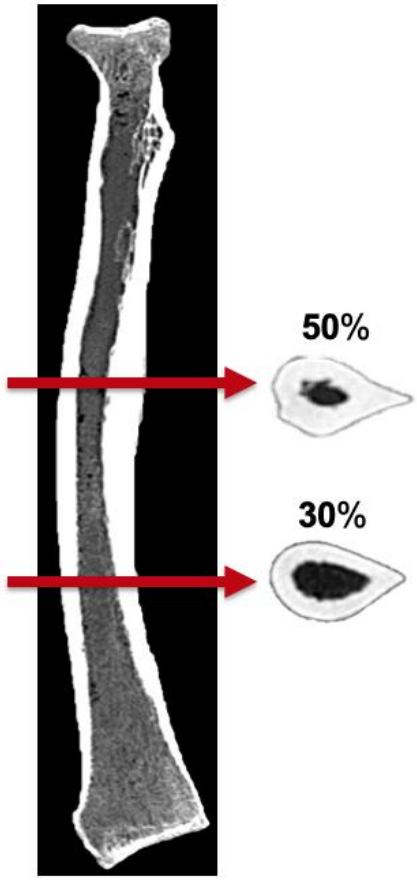

Figure 1. Segmentation Sites. A depiction of the two VOIs (30%, 50%) segmented for further analyses.

Total Ct.Th and vBMD Calculations

Total Ct.Th for both the 30% and 50% VOIs was calculated by defining the cortex between the periosteal and endosteal borders and averaging the multiple Ct.Th measurements cross the entire volume of interest (Figure 2), giving a total Ct.Th measurement per VOI (30%, 50%). vBMD for both the 30% and 50% VOIs was calculated through the formation of calibration curves (Figure 3) using grey scale values to threshold cortical bone (175-255 mg/cm^3) and calculate the Hounsfield Units (HU). Calibration curves were created from the known densities (200-800 mg/cm^3) found in the QRM phantoms and saline syringe placed in each scan (Figure 4). This method produced a total BMD per VOI (30% and 50%).

Figure 2. Total Ct.Th Calculation. A depiction of how total Ct.Th was calculated within the cross-section. The red line shows where Ct.Th was calculated at a single point, and this process was done at various points around the cortex automatically via SkyScan. The various Ct.Th measurements were then averaged to obtain total Ct.Th.

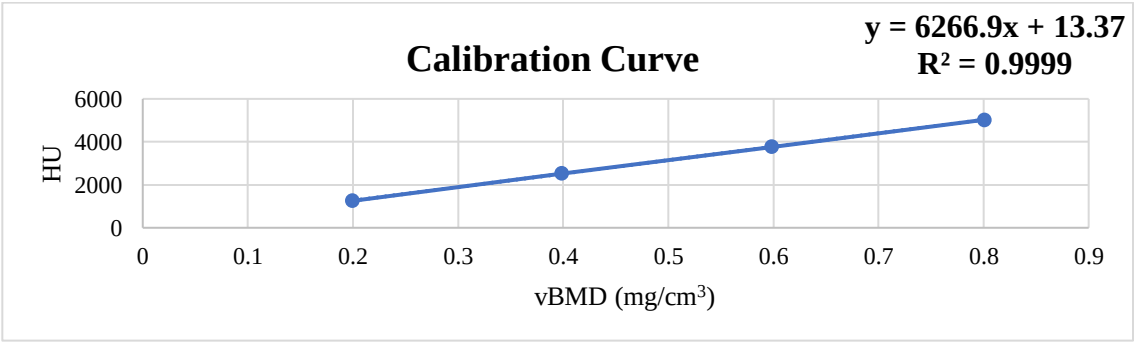

Figure 3. Calibration Curve Using Hounsfield Units. The above calibration curve is an example of how Hounsfield units (Hu) were used to calculate vBMD for each VOI.

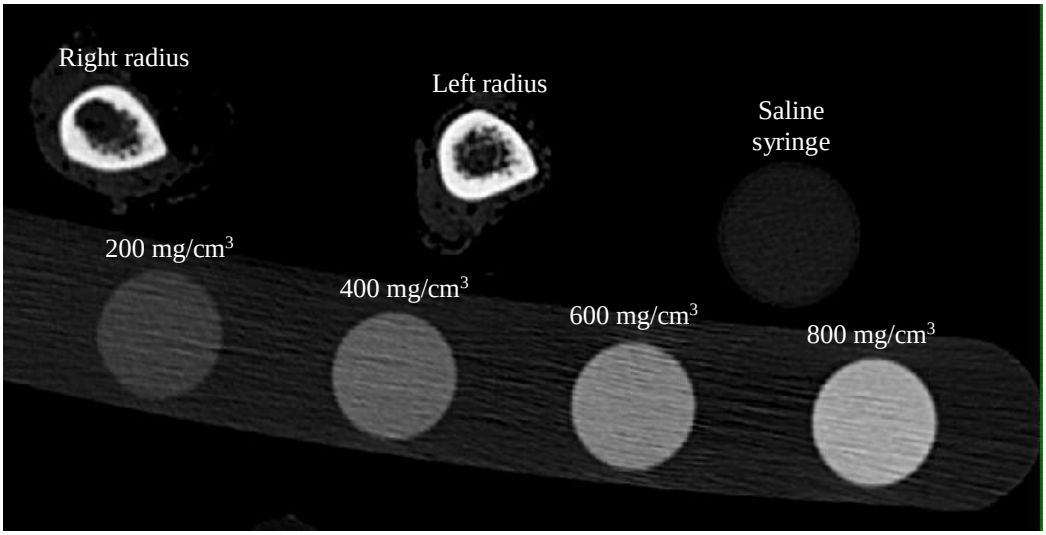

Figure 4. CT Image with QRM Cortical Phantoms, Radius Cross-sections, and Saline Syringe. The above CT image illustrates the placement of the QRM phantom of known densities (200-800 mg/cm^3) and saline syringe. The saline syringe was used to calibrate the QRM phantom densities, and the QRM phantom known densities were used in the formation of calibration curves in order to calculate total vBMD for each VOI (30%, 50%).

Cross-sectional Variation in Ct.Th and vBMD

In addition to the total values described above, Ct.Th and vBMD measurements were also taken within a single cross-section for each radii in the study using similar SkyScan methods as mentioned above. Using the cross-sectional images obtained during segmentation, custom regions of interest (ROIs) were created for each CT scan with respect to anatomical positions around the cortex: anterior, posterior, medial and lateral. In order to define the center point of the scan, the diameter of the medullary cavity was measured both horizontally and vertically (Figure 5). These diameter measurements (in pixels) were then divided in half to determine a center coordinate point for the cross-section, which was used as the universal reference point for placing the custom ROIs around each cross-section. It is important to note that the horizonal center point was always used as the reference point when initiating the measurement of the medullary cavity diameter vertically. In order to control for the total size of the cross-section

with relationship to the cortical area of the custom ROI, total horizontal diameter of the cross-section, from the medial (interosseous) crest to lateral border, was measured, and the width of the custom ROI was adjusted to be 10% of the total diameter (Figure 6).

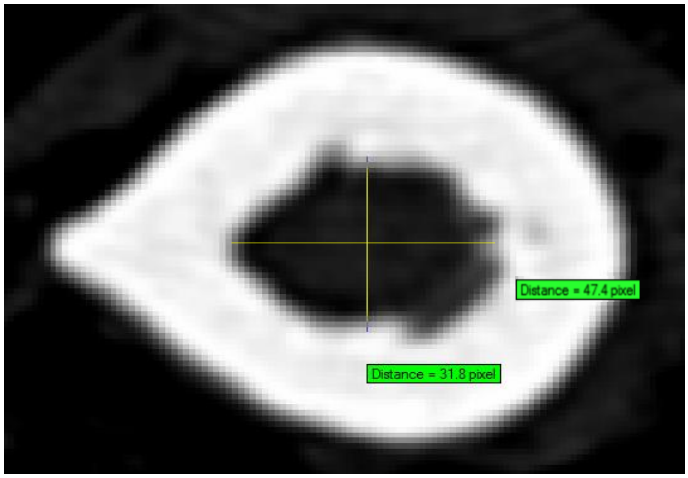

Figure 5. Cross-sectional Diameter Measurements.
A depiction of how the diameter of the medullary cavity, both horizontally and vertically, was measured in SkyScan to determine the anatomical center of the cross-section.

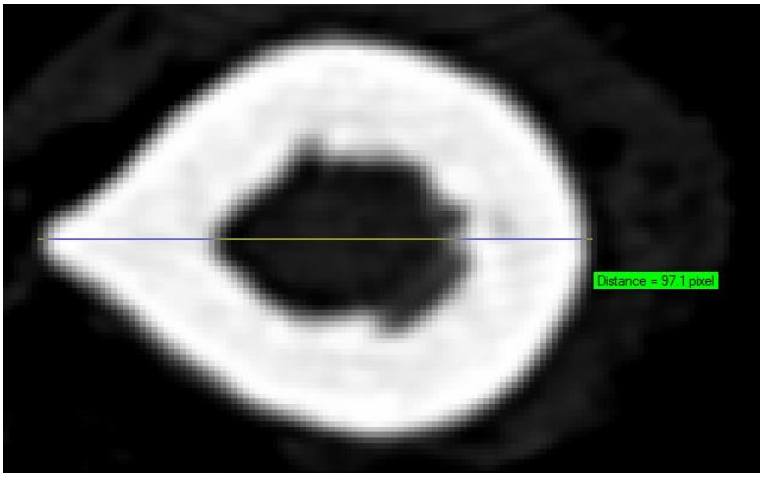

Figure 6. Cross-sectional Size Adjustment Measurements.
A depiction of how the total diameter of the cross-section was measured, from interosseous crest to lateral border, in SkyScan.

The custom regions of interest (ROIs) were normalized to the size of each radius and each VOI with respect to the total size of the cross-section. Once the location of the ROIs was set, it was kept consistent between all anatomical measurements around the cross-section. Thresholding values were also kept consistent in order to identify cortical bone. The custom ROIs were placed at the center of the anterior, posterior, medial, and lateral locations around the cortex, as demonstrated in Figure 7. Within these custom ROIs, cortical thickness (Ct.Th) and volumetric bone mineral density (vBMD) measurements were calculated using standard protocols in SkyScan as described above.

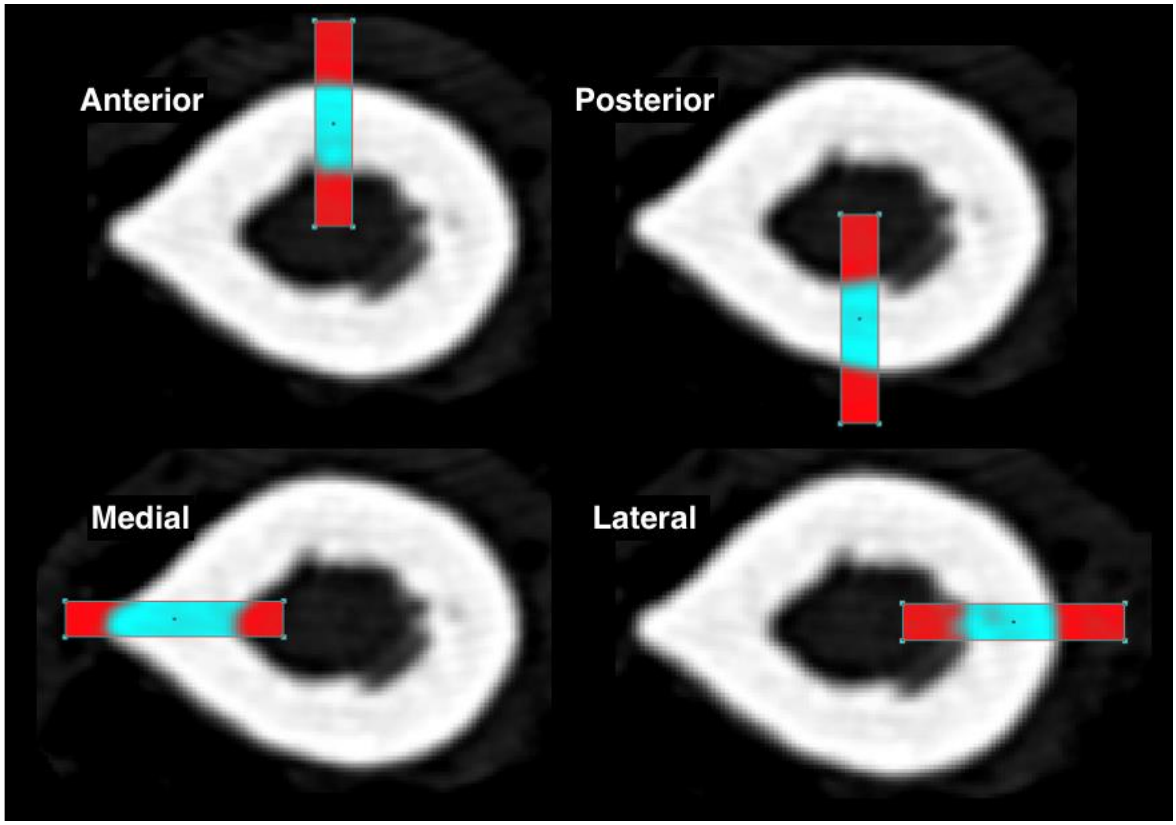

Figure 7. Custom Regions of Interest. A depiction of the anatomically-specific custom ROIs.

Statistical Analysis

Two-sample t-tests were conducted to see if differences in Ct.Th or vBMD were present between males and females. Sex-specific linear regression analyses were conducted with age as the predictor variable and cortical thickness (Ct.Th) and volumetric bone mineral density (vBMD) as the outcome variables in order to evaluate the effects of age on Ct.Th and vBMD variation. In order to quantify the variation present along the radial diaphysis, paired samples t-tests compared both Ct.Th and vBMD between the two sites of interest (30%, 50%). ANOVA with Bonferroni post-hoc tests were used to compare the anatomically-specific means of Ct.Th and vBMD measurements (anterior, posterior, medial, lateral) to each other within a single VOI, as well as to compare a single ROI between the two VOIs (30%, 50%). Lastly, Pearson's correlation coefficients were used to investigate the relationships between both Ct.Th and vBMD to aBMD (α=0.05). All statistical analyses were conducted in SPSS v(25).

Results

Sex Differences in Total Ct.Th and vBMD

In order to determine if differences were present in both Ct.Th and vBMD between males and females, two-sample t-tests were conducted. They revealed that there were significant sex differences in Ct.Th at both VOIs (30%, 50%) ($p<0.0001$), but only for 30% vBMD ($p=0.04$). The comparisons of total Ct.Th and vBMD between sexes are further illustrated in Table 1 as well as in Figures 8 & 9.

	Males Mean±SD	Females Mean±SD	p
Ct.Th			
30%	2.5±0.5	1.6±0.5	**<0.0001**
50%	2.8±0.6	1.8±0.6	**<0.0001**
vBMD			
30%	1110.0±24.3	1096.0±25.5	**0.04**
50%	1101.3±24.5	1086.2±34.4	0.06

Table 1. Comparisons of Ct.Th and vBMD Between Sexes. This table illustrates the significant differences in Ct.Th and vBMD between sexes. Additionally, it can be seen that males are larger than females in both parameters (Ct.Th and vBMD), and at both sites.

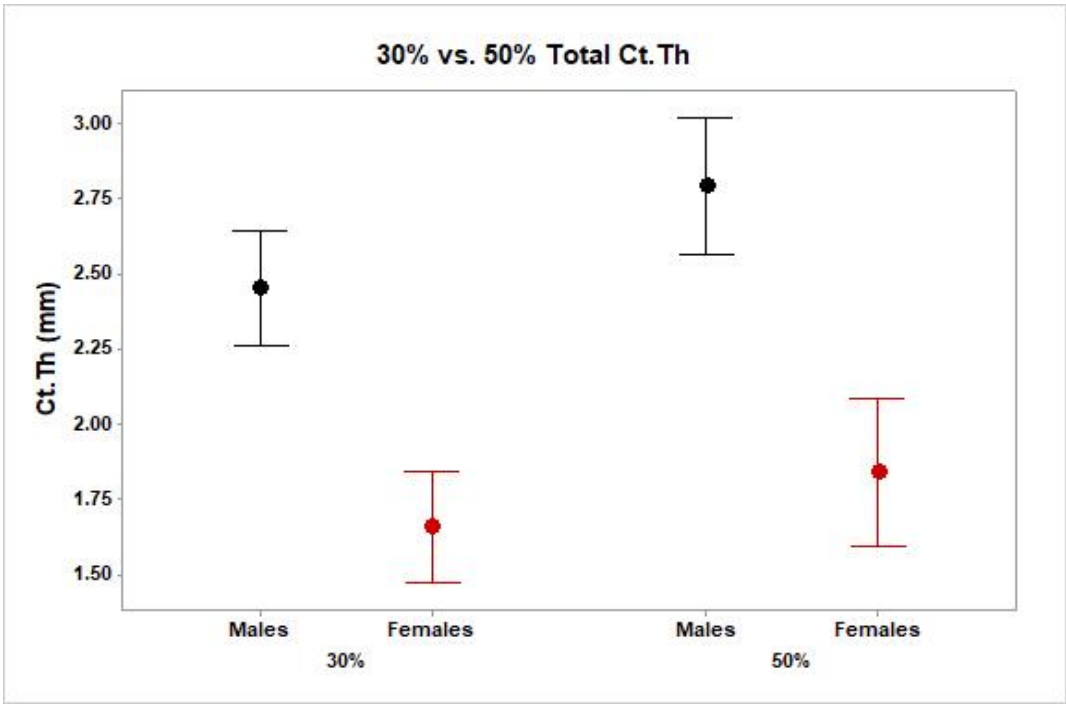

Figure 8. Males versus Females Ct.Th Interval Plot. The above interval plot depicts the significant differences in Ct.Th between males and females at both the 30% and 50% VOIs, with males having significantly larger Ct.Th values than females.

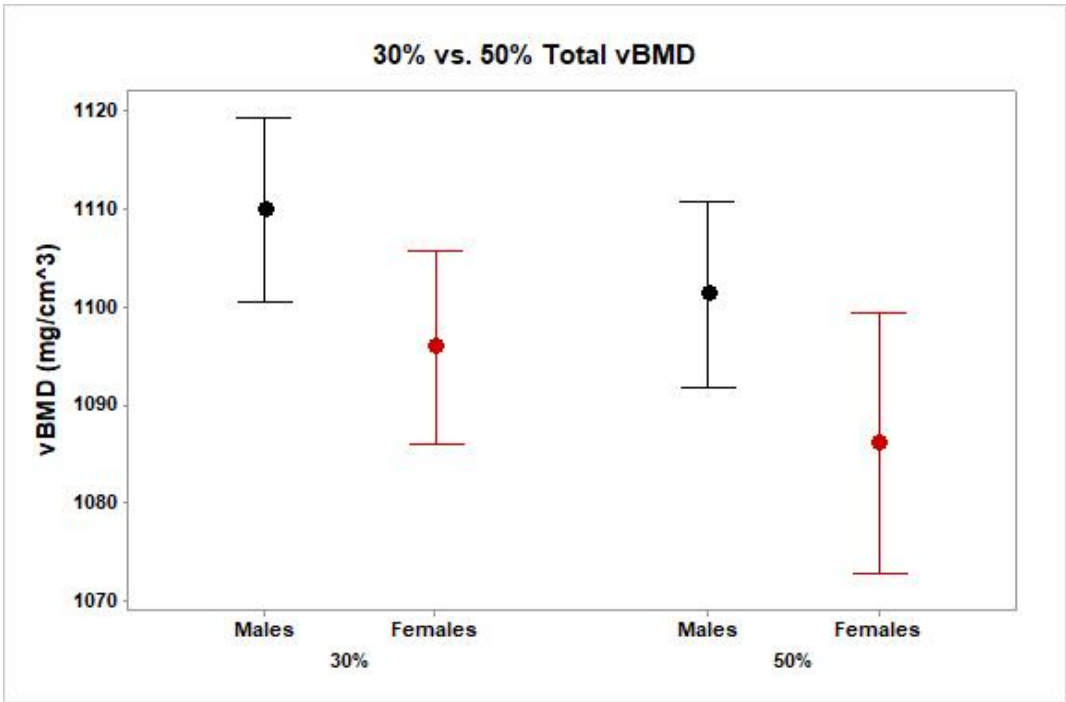

Figure 9. Males versus Females vBMD Interval Plot. The above interval plot depicts the significant differences in vBMD between males and females at the 30% VOI, along with the insignificant differences in vBMD found between sexes at the 50% VOI.

Differences in Whole Ct.Th and vBMD Between VOIs (30%, 50%)

It was hypothesized that there would be significant differences in whole Ct.Th and vBMD along the diaphysis of the radius. This was tested by comparing the Ct.Th and vBMD values between the 30% and 50% VOIs. A paired-samples t-test revealed there were significant differences in both Ct.Th and vBMD between VOIs. More specifically, total Ct.Th at the 50% VOI was significantly larger than at the 30% for both males ($p<0.0001$) and females ($p=0.001$), possibly suggesting the 30% VOI may be at a higher risk for fracture. Conversely, total vBMD was significantly higher at the 30% VOI compared to the 50% in both males ($p=0.002$) and females ($p=0.006$). This relationship is further illustrated in Table 2 and Figures 10 & 11.

	30% Mean±SD	50% Mean±SD	p
Males			
Ct.Th	2.5±0.5	2.8±0.6	**<0.0001**
vBMD	1110.0±24.3	1101.3±24.5	**0.002**
Females			
Ct.Th	1.6±0.5	1.8±0.6	**0.001**
vBMD	1096.0±25.5	1086.2±34.4	**0.006**

Table 2. Comparisons of Ct.Th and vBMD Between VOIs. In the above table, the p-values indicate significant differences in Ct.Th and vBMD between VOIs (30%, 50%) for both males and females.

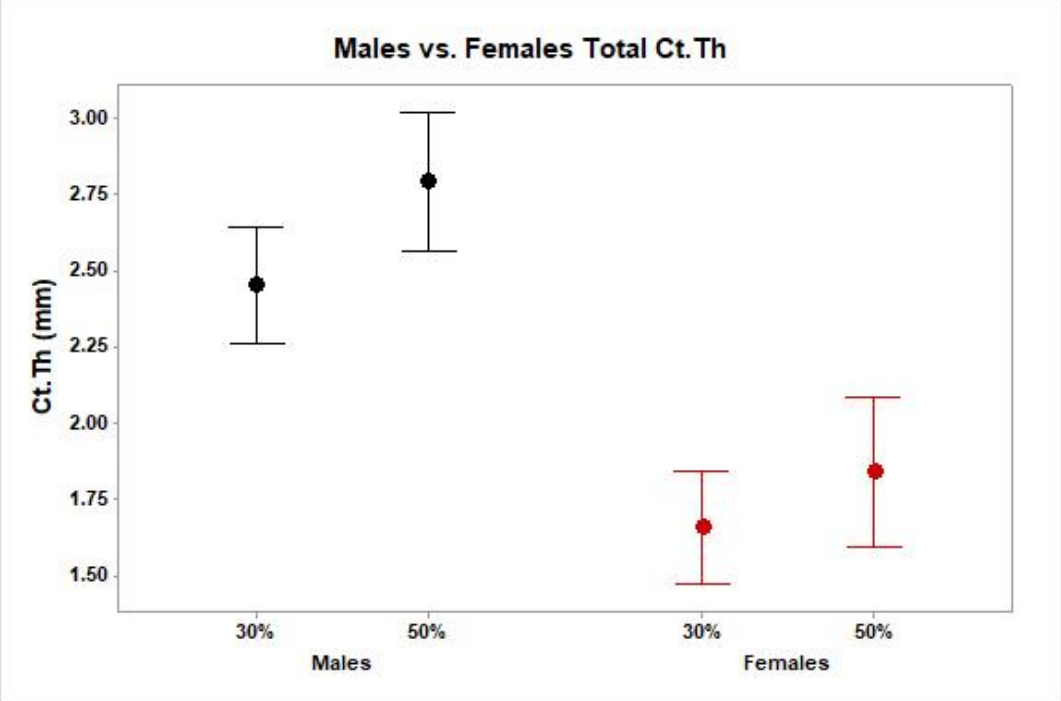

Figure 10. 30% versus 50% Ct.Th Interval Plot. The above interval plot depicts the significant differences in Ct.Th between VOIs (30%, 50%) for both males and females, with Ct.Th significantly larger at the 50% VOI for both sexes.

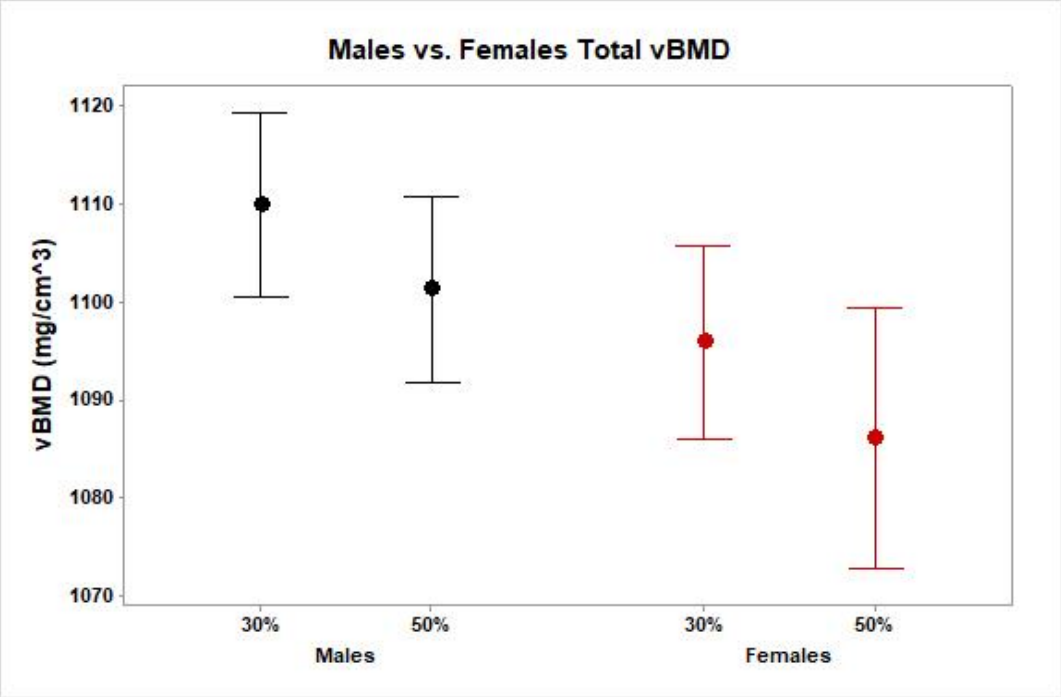

Figure 11. 30% versus 50% vBMD Interval Plot. The above interval plot depicts the significant differences in vBMD between VOIs (30%, 50%) for both males and females, with vBMD significantly larger at the 30% VOI for both sexes.

Sex-specific Linear Regressions

In order to investigate changes in both Ct.Th and vBMD with age, sex-specific linear regressions were used. The linear regression plots with age versus 30% or 50% Ct.Th found no significant changes in Ct.Th with age for either males (p=0.053, p=0.19) or females (p=0.083, p=0.10) (Figures 12 & 13); however, it is important to note there was a general decreasing trend in Ct.Th with age for both sexes. The linear regression plots with age versus 30% or 50% vBMD ultimately revealed no significant changes in vBMD with age for either males (p=0.60, p=0.48) or females (p=0.99, p=0.96) (Figures 14 & 15).

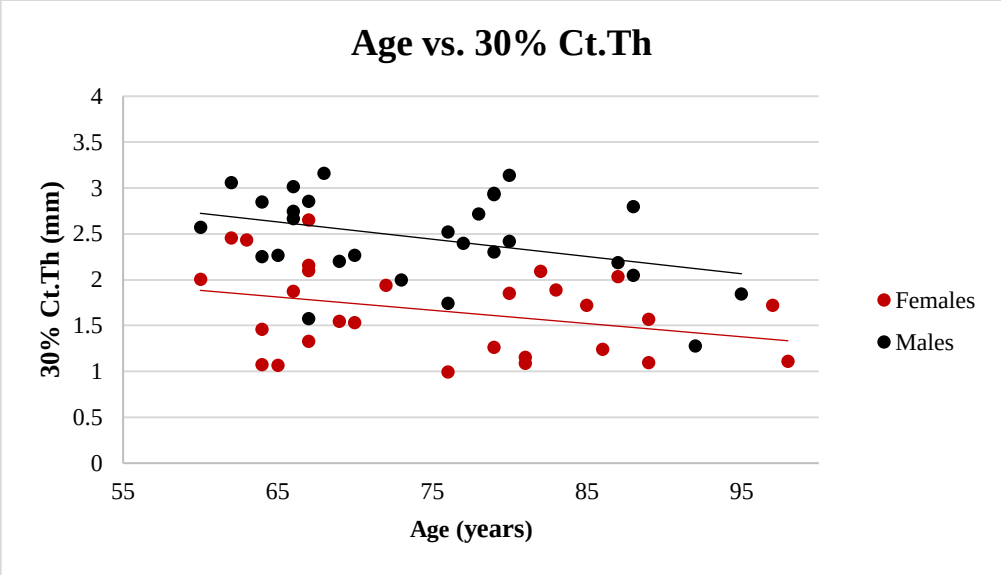

Figure 12. Linear Regression of Age versus Ct.Th. There was no significant linear relationship between age and Ct.Th at the 30% VOI for either males (p=0.053, R^2=0.14) or females (p=0.083, R^2=0.11); however, a decreasing trend in Ct.Th can be observed for both sexes.

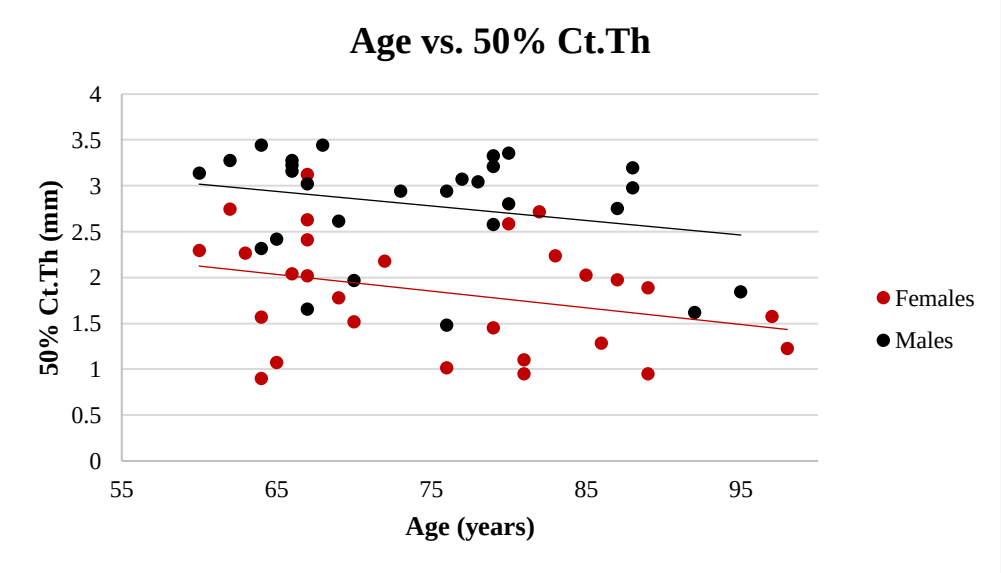

Figure 13. Linear Regression of Age versus 50% Ct.Th. There was no significant linear relationship between age and Ct.Th at the 50% VOI for either males (p=0.19, R^2=0.07) or females (p=0.10, R^2=0.10); however, a decreasing trend in Ct.Th can be observed for both sexes.

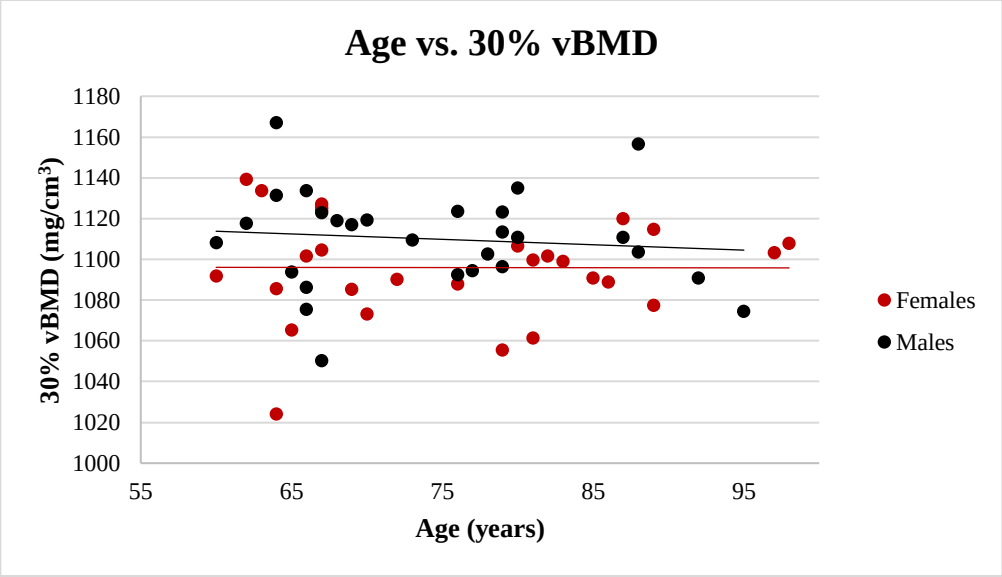

Figure 14. Linear Regression of Age versus 30% vBMD. There was no significant linear relationship between age and vBMD at the 30% VOI for either males ($p=0.60$, $R^2=0.01$) or females ($p=0.99$, $R^2<0.0001$).

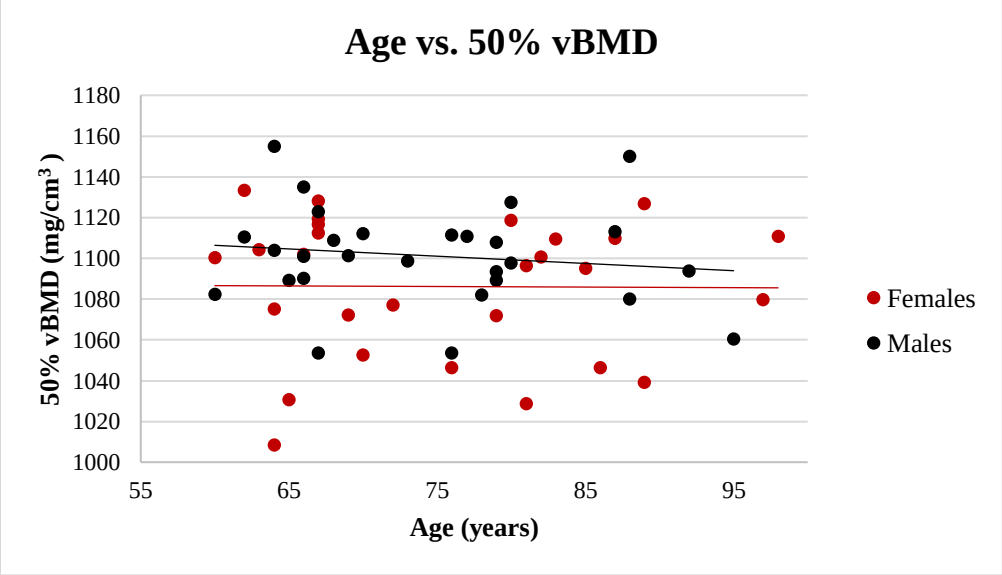

Figure 15. Linear Regression of Age versus 50% vBMD. There was no significant linear relationship between age and vBMD at the 50% VOI for either males ($p=0.48$, $R^2=0.02$) or females ($p=0.96$, $R^2<0.0001$).

Differences in Ct.Th and vBMD Between ROIs Within a Single VOI

The first step in moving to analyses within the cross-section was to test for differences between the four anatomically-defined ROIs (anterior, posterior, medial, lateral) within each of the VOIs (30%, 50%). One-way ANOVAs demonstrated no significant differences in vBMD between ROIs within either the 30% or 50% VOI for either sex ($p>0.05$) (Figures 16 & 17); however, significant differences in Ct.Th between ROIs displayed sex-specific patterns within each VOI (Figures 18 & 19). Medial Ct.Th was significantly larger than lateral Ct.Th at both the 30% and 50% VOIs for both males ($p=0.001$, $p<0.0001$) and females ($p=0.027$, $p=0.025$). At the 50% VOI for males, the medial ROI was also significantly larger than the anterior and posterior ROIs ($p=0.006$, $p=0.042$). Table 3 further illustrates the differences between each ROI within a single VOI and provides specific p-values for each comparison.

Sex	Location	Comparison Locations	p
30% Ct.Th			
Males	Anterior	Posterior	1.000
		Medial	0.146
		Lateral	0.728
	Posterior	Anterior	1.000
		Medial	0.177
		Lateral	0.624
	Medial	Anterior	0.146
		Posterior	0.177
		Lateral	**0.001**
	Lateral	Anterior	0.728
		Posterior	0.624
		Medial	**0.001**
Females	Anterior	Posterior	1.000
		Medial	0.060
		Lateral	1.000
	Posterior	Anterior	1.000
		Medial	0.227
		Lateral	1.000
	Medial	Anterior	0.060
		Posterior	0.227
		Lateral	**0.027**
	Lateral	Anterior	1.000
		Posterior	1.000
		Medial	**0.027**
50% Ct.Th			
Males	Anterior	Posterior	1.000
		Medial	**0.006**
		Lateral	0.686
	Posterior	Anterior	1.000
		Medial	**0.042**
		Lateral	0.173
	Medial	Anterior	**0.006**
		Posterior	**0.042**
		Lateral	**<0.0001**
	Lateral	Anterior	0.686
		Posterior	0.173
		Medial	**<0.0001**
Females	Anterior	Posterior	1.000
		Medial	0.552
		Lateral	1.000
	Posterior	Anterior	1.000
		Medial	0.672
		Lateral	1.000
	Medial	Anterior	0.552
		Posterior	0.672
		Lateral	**0.025**
	Lateral	Anterior	1.000
		Posterior	1.000
		Medial	**0.025**

Sex	Location	Comparison Locations	p
30% vBMD			
Males	Anterior	Posterior	1.000
		Medial	1.000
		Lateral	1.000
	Posterior	Anterior	1.000
		Medial	1.000
		Lateral	1.000
	Medial	Anterior	1.000
		Posterior	1.000
		Lateral	1.000
	Lateral	Anterior	1.000
		Posterior	1.000
		Medial	1.000
Females	Anterior	Posterior	1.000
		Medial	0.210
		Lateral	1.000
	Posterior	Anterior	1.000
		Medial	1.000
		Lateral	1.000
	Medial	Anterior	0.210
		Posterior	1.000
		Lateral	1.000
	Lateral	Anterior	1.000
		Posterior	1.000
		Medial	1.000
50% vBMD			
Males	Anterior	Posterior	1.000
		Medial	1.000
		Lateral	1.000
	Posterior	Anterior	1.000
		Medial	1.000
		Lateral	1.000
	Medial	Anterior	1.000
		Posterior	1.000
		Lateral	1.000
	Lateral	Anterior	1.000
		Posterior	1.000
		Medial	1.000
Females	Anterior	Posterior	1.000
		Medial	1.000
		Lateral	1.000
	Posterior	Anterior	1.000
		Medial	1.000
		Lateral	1.000
	Medial	Anterior	1.000
		Posterior	1.000
		Lateral	1.000
	Lateral	Anterior	1.000
		Posterior	1.000
		Medial	1.000

Table 3. Results of ANOVAs Comparing ROIs Within a Single VOI. The above table illustrates the specific p-values associated with each comparison between ROIs within a single VOI for both Ct.Th and vBMD.

Differences in Ct.Th and vBMD Between VOIs at a Single ROI

The second portion of the cross-sectional analysis consisted of analyzing possible differences between VOIs (30%, 50%) at a single ROI (anterior, posterior, medial, lateral). For example, the anterior ROI was compared between the 30% and 50% VOIs. This process was completed for each of the four ROIs for both sexes using paired t-tests for statistical analysis. For both sexes, differences in vBMD between VOIs were identified at only the medial ROI ($p<0.01$) (Figure 16 & 17). Significant differences in Ct.Th between VOIs were found at all ROIs for females, and at all but the lateral ROI for males ($p<0.01$) (Figures 18 & 19). Table 4 further illustrates the differences between VOIs at a single ROI and provides specific p-values for each comparison.

	30% Ct.Th Mean±SD	50% Ct.Th Mean±SD	p
Anterior			
Males	0.97±0.11	1.1±0.12	**<0.0001**
Females	0.73±0.12	0.84±0.15	**<0.0001**
Posterior			
Males	0.99±0.12	1.1±0.16	**<0.0001**
Females	0.73±0.12	0.84±0.14	**<0.0001**
Medial			
Males	1.1±0.11	1.2±0.15	**<0.0001**
Females	0.78±0.11	0.93±0.17	**<0.0001**
Lateral			
Males	0.96±0.11	1.0±0.15	0.06
Females	0.69±0.12	0.77±0.17	**0.006**

	30% vBMD Mean±SD	50% vBMD Mean±SD	p
Anterior			
Males	1107.2±27.3	1107.9±28.5	0.90
Females	1095.6±28.7	1089±36.5	0.36
Posterior			
Males	1098.6±34.8	1097.0±34.0	0.72
Females	1088.4±29.9	1086.9±37.2	0.80
Medial			
Males	1109.9±29.2	1089.8±27.6	**<0.0001**
Females	1095.0±38.1	1078.3±41.8	**0.02**
Lateral			
Males	1101.1±31.2	1096.6±36.1	0.35
Females	1091.1±25.6	1076.8±63.3	0.18

Table 4. Results of ANOVAs Comparing a Single ROI Between VOIs. The above table illustrates the specific p-values associated with each comparison between VOIs at a single ROI for both Ct.Th and vBMD.

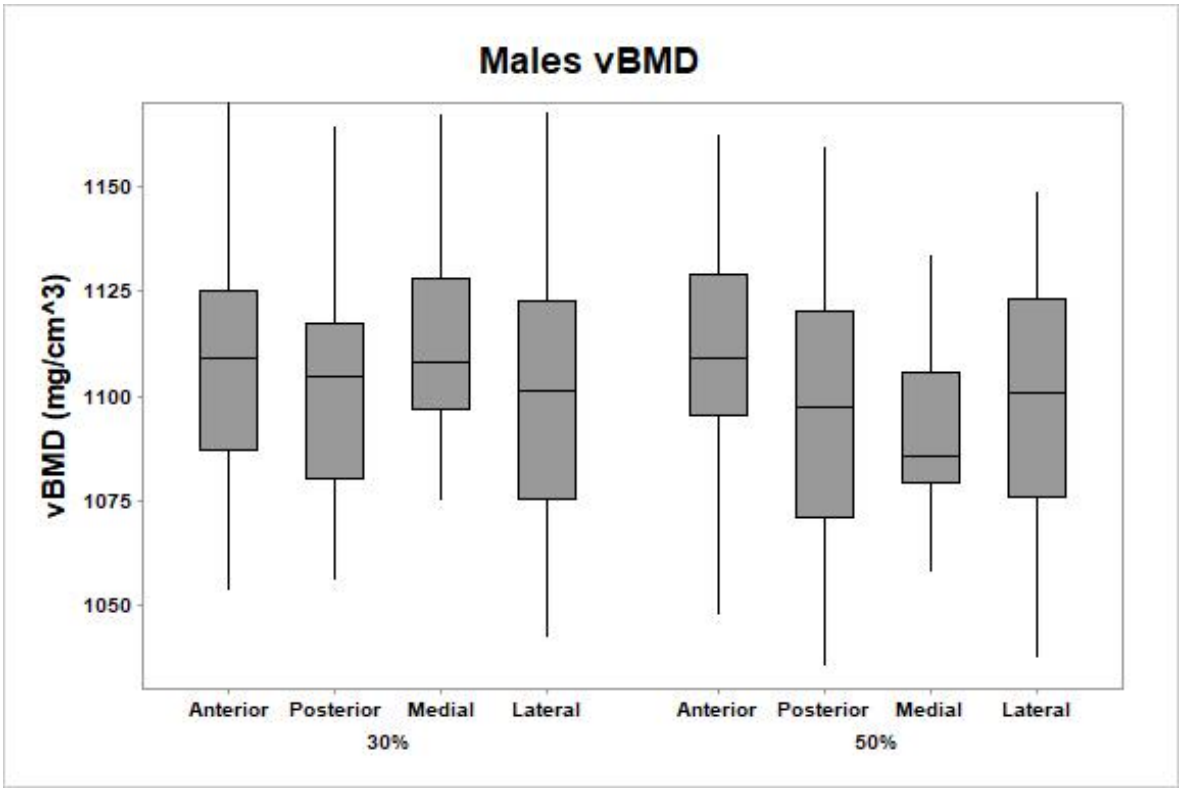

Figure 16. Boxplot of Males vBMD. The above boxplot demonstrates there were no significant differences in vBMD found between ROIs within a VOI (30%, 50%) for males. Additionally, when comparing a single anatomical ROI between VOIs, only the 30% medial vBMD was significantly larger than the 50% medial vBMD in males ($p<0.01$).

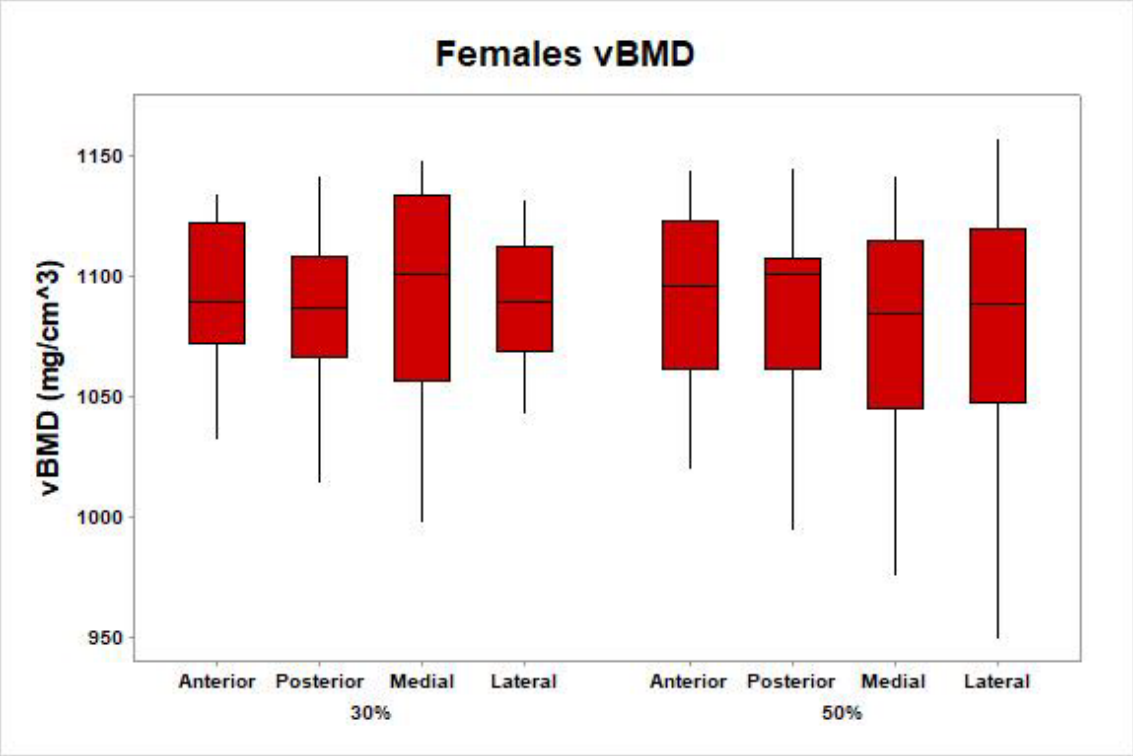

Figure 17. Boxplot of Females vBMD. The above boxplot demonstrates there were no significant differences in vBMD found between ROIs within a VOI (30%, 50%) for females. Additionally, when comparing a single ROI between VOIs, only the 30% medial vBMD was significantly larger than the 50% medial vBMD in females (p=0.02).

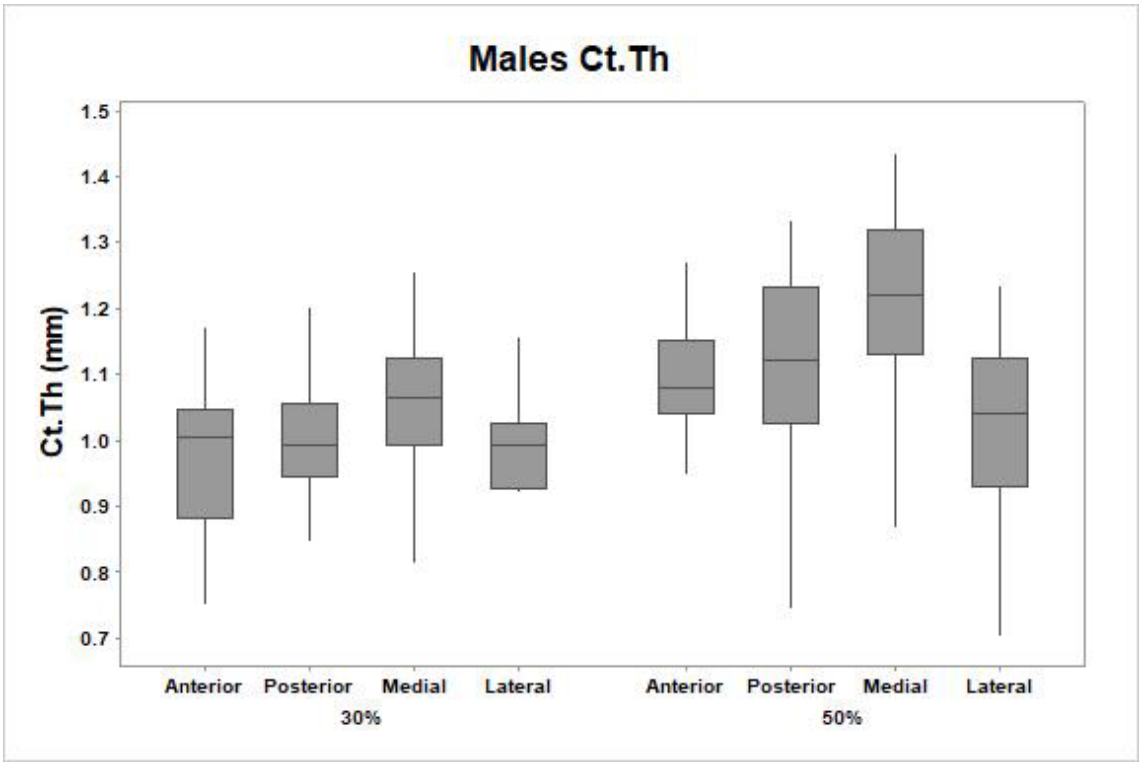

Figure 18. Boxplot of Males Ct.Th. The above boxplot demonstrates that medial Ct.Th was significantly larger than lateral Ct.Th at both the 30% and 50% VOIs ($p<0.05$). At the 50% VOI for males, the medial ROI was also significantly larger than the anterior and posterior ROIs ($p<0.05$). Between VOIs at a single ROI, males had significant differences in Ct.Th at the anterior, posterior, and medial ROIs ($p<0.01$), with the 50% VOI being larger than the 30% at each ROI.

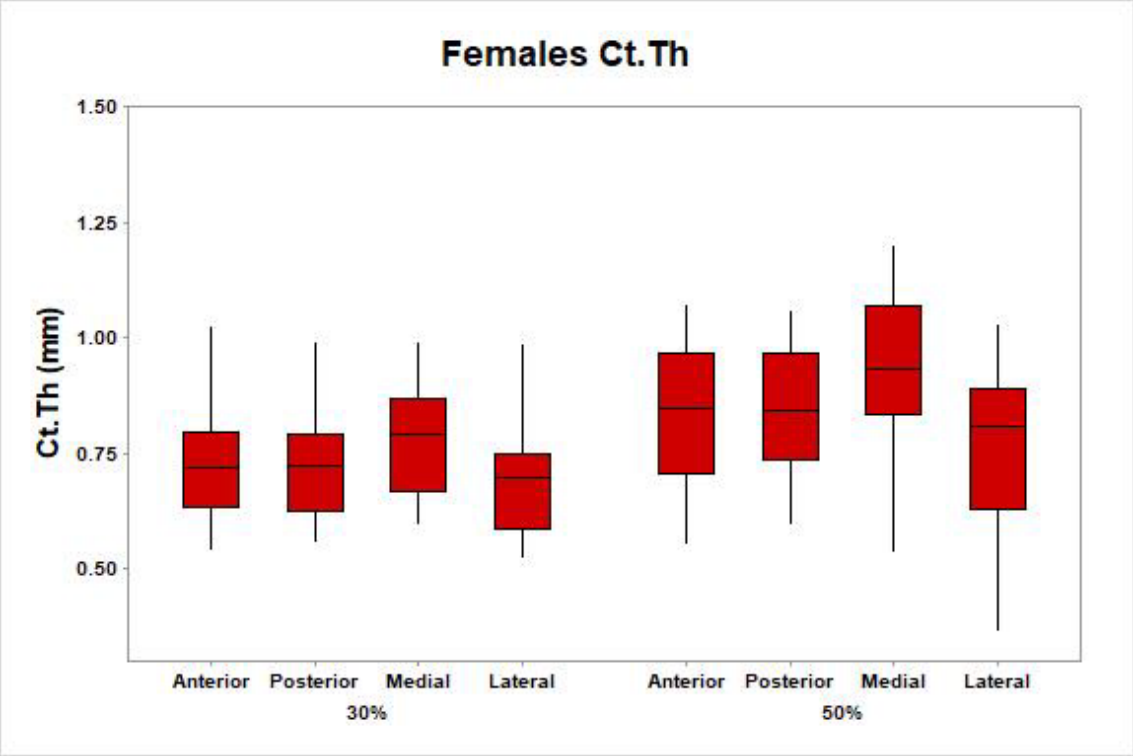

Figure 19. Boxplot of Females Ct.Th. The above boxplot demonstrates that medial Ct.Th was significantly larger than lateral Ct.Th at both the 30% and 50% VOIs for females ($p<0.05$). Between VOIs at a single ROI, females had significant differences in Ct.Th at all of the ROIs (anterior, posterior, medial, lateral) ($p<0.01$).

Relationships in Ct.Th and vBMD with aBMD

In a sub-sample of 16 males and 26 females, statistically significant positive correlations were found for both Ct.Th and vBMD compared to aBMD in females ($p<0.05$), but only between Ct.Th and aBMD in males ($p<0.05$) (Figures 20 & 21).

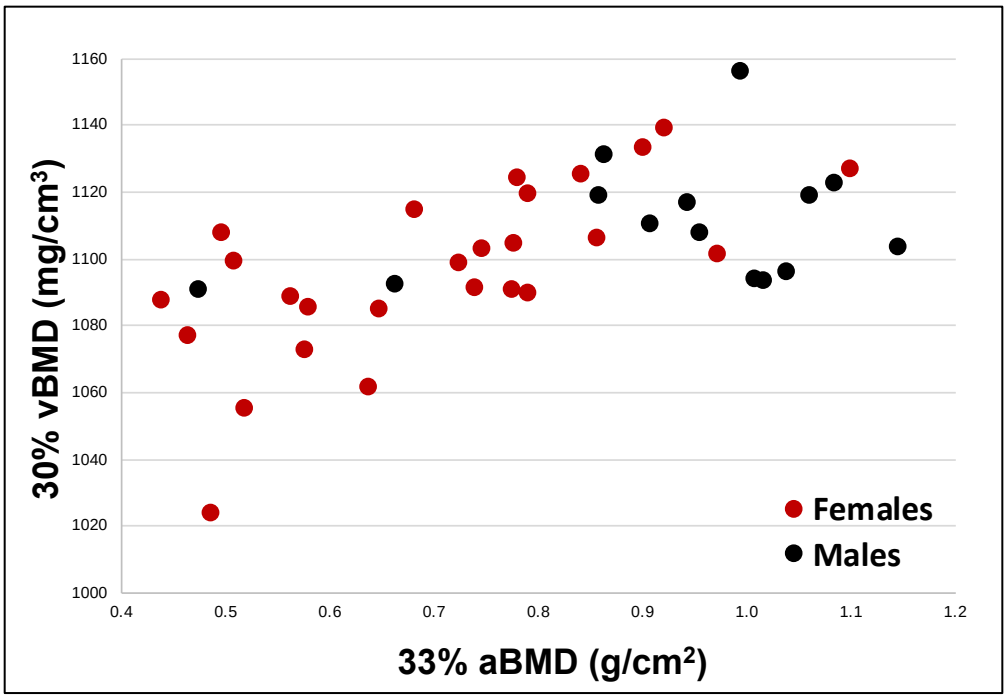

Figure 20. Pearson's Correlation 33% aBMD versus 30% vBMD. The above Pearson's correlation shows there are positive relationships between aBMD and vBMD for males ($p=0.047$) and females ($p<0.0001$).

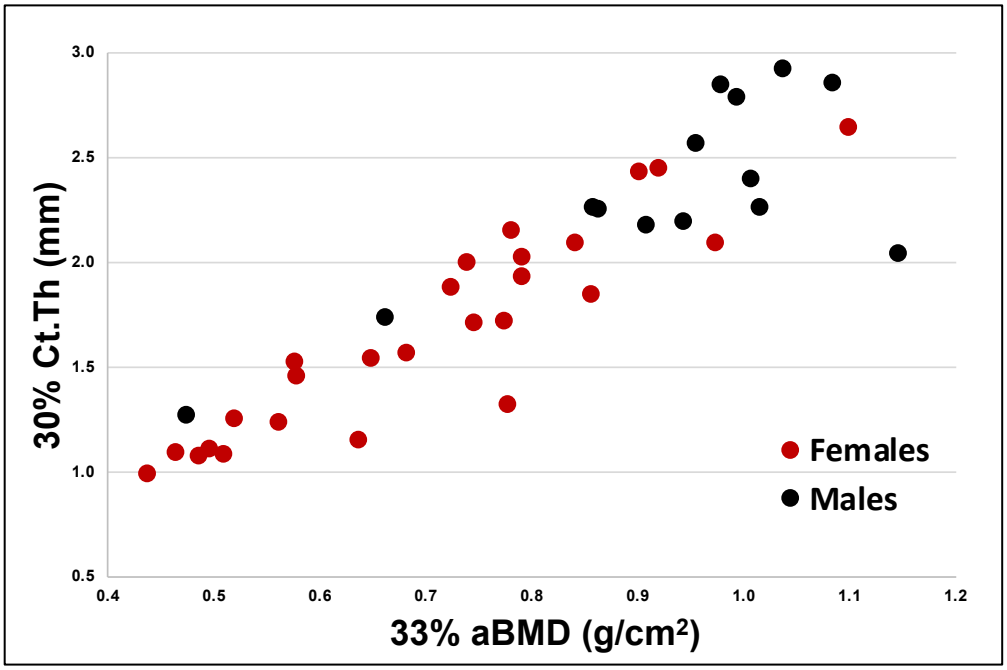

Figure 21. Pearson's Correlation 33% aBMD versus 30% Ct.Th. The above Pearson's correlation shows there are positive relationships between aBMD and Ct.Th for males ($p<0.0001$) and females ($p<0.0001$).

Discussion

The present work demonstrates variance in Ct.Th both along the radial diaphysis and within a single cross-section and suggests that an average, or total, value may not necessarily provide a biologically meaningful measurement of bone quality. Due to smaller amounts of variation found in vBMD within the radius, both for males (1050-1167 mg/cm^3) and females (1024-1139 mg/cm^3), this measurement may not represent a comprehensive evaluation of fracture risk.

With the goal of understanding skeletal variation, research has been done investigating differences in bone loss with respect to subject-level variables, such as age. Dalzell (2009) conducted an experiment centered around determinants of strength in both the distal radius and tibia. They recruited a sample of 58 males and 74 females ranging from 20 to 79 years of age from a primary care medical practice in order to investigate age-related changes on the micro-architecture and strength of upper and lower limb bones. Through the use of peripheral quantitative computed tomography (pQCT), they were able to determine that in females, the largest effects of age can be seen with Ct.Th and vBMD in both the radius and tibia, where both parameters declined with age. When performing the same analyses in males, the effects were found with all parameters except Ct.Th. While declines in Ct.Th and vBMD with age have been found in robust samples with diverse age ranges, it was of interest to explore this relationship strictly in a population of subjects over the age of 60 in order to see if Ct.Th and vBMD declines are still significant after the age of 60. Although established age differences in the radius were found in previous studies, the current study did not observe significant declines in Ct.Th or vBMD with age; however, a decreasing trend in Ct.Th was seen with age in this study even though it was not statistically significant. This is likely due the strictly elderly sample used,

rather than a well-rounded sample including individuals of all ages. Additionally, although significant relationships between Ct.Th and vBMD with age were observed in both the radius and tibia in other studies (Ho-Pham et al., 2018), age only explained 27.2% and 34.9% of the variation in vBMD in Dalzell and colleagues' (2009) study, leaving a large amount of variation unexplained.

Another subject-level variable that has been used in previous work to explain bone loss is sex. A study by Naganathan (2003) examined sex differences in bone mineral content (BMC), areal bone mineral density (aBMD), and volumetric bone mineral density (BMD) at three sites of interest (the third lumbar vertebra, femoral neck, and forearm (1/3 radius) by comparing opposite sex-twins. Specifically, BMC was significantly higher in males at all three sites, aBMD was significantly higher in males at all sites except the spine, and vBMD was significantly higher in females at all sites except for the radius. Ultimately, there was no evidence that estimated vBMD predicted fracture or bone strength better than aBMD, and the opposite trends for aBMD and vBMD were hypothesized by Naganathan and colleagues to be caused by a function of the formula used to calculate vBMD (BMC divided by volume). They concluded that radial aBMD and BMC values between males and females significantly differed ($p<0.001$); however, radial vBMD did not demonstrate significant differences between sexes ($p=0.42$). Ho-Pham and colleagues (2018) also discovered sex differences in bone architecture and bone fragility in the radius between sexes. They found that in the radius, age-related reduction in cortical vBMD was greater in females than males, but the reduction in trabecular vBMD was comparable between sexes. Bonel (2004) conducted a study in which they investigated relationships between region-specific cortical parameters at the distal radius with respect to sex, age, and osteoporotic status as determined through the use of CT. In order to accomplish this objective, they obtained bone

mineral content (BMC) values (in grams) using DXA and used a digital image analysis algorithm to characterize bone density and cortical structure at the distal radial metaphysis. Ultimately, they determined that the estimated strength of the distal radius, using finite element modeling, was significantly lower in women than in men, and DXA BMC values displayed a similar sex difference, with BMC being significantly lower in women than in men. They also found that women exhibited significantly lower cortical area and cortical density.

The current work aimed to explore variation in Ct.Th and vBMD as it related to both sex and age. It was found that age only explained 1% of the variation in 30% vBMD in males and less than 0.1% in females. At the 50% site, age was only able to predict 2% of the variation in vBMD in males and less than 0.1% in females. In terms of Ct.Th, age explained only 14% of the variation in 30% Ct.Th in males and only 11% in females. At the 50% VOI, age was only able to predict 7% of the variation in Ct.Th in males and 10% in females. Therefore, it is evident that other factors, beyond age, may be affecting Ct.Th and vBMD. Age likely explains more of the variation in males because they experience fewer confounding circumstances, besides age, related to bone loss than females. In terms of sex differences, the current study found significant differences between males and females for Ct.Th at both VOIs ($p<0.0001$), but only for vBMD at the 30% VOI ($p=0.04$). Ultimately, it is important to consider the significant biological differences found between males and females in order to understand bone quality changes.

A study conducted by Hunter et al. (2017) explored variation along the diaphysis of a single element. More specifically, they investigated the co-variation between geometric and material properties, specifically cortical area (Ct.Ar), section modulus (Z), and volumetric bone mineral density (vBMD), present along the tibial diaphysis. While their study focused on co-variances of geometric and material properties within the tibia, a weight bearing bone, significant

differences ($p<0.001$) were quantified in Ct.Ar, Z, and vBMD between all VOIs. Considering the relationships found in a weight bearing bone (tibia), it was of interest to the current study in order to see if the same principles applied to a non-weight bearing bone (radius) in comparison to the tibia. Ultimately, the results of the current study found a similar inverse relationship between Ct.Th and vBMD, with Ct.Th being significantly greater at the 50% volume of interest (VOI) and vBMD being significantly greater at the 30% VOI for both sexes. This inverse phenomenon demonstrates the functional adaptation of the radius where a smaller thickness of bone (Ct.Th) may be compensated for by an increase in mineralization (vBMD) depending on the site along the radial diaphysis (Hunter et al., 2017; Jepsen, 2011). In terms of fracture risk, this likely implies that areas of bone with lower Ct.Th are more susceptible to fracture, and bone is functionally attempting to prevent this fracture by increasing mineralization in that vulnerable area.

Skeletal variation can also be assessed at the cross-sectional level. Although there have been studies examining the differences within a single cross-section of the femur, there have not yet been any studies investigating such a phenomenon in the radius. Yang (2014) investigated variances within a single cross-section of the femur by taking measurements at four anatomical quadrants around the cortex: inferoanterior, inferoposterior, superoanterior, and superoposterior. Their sample included postmenopausal women and measured both cortical thickness (Ct.Th) and volumetric bone mineral density (vBMD) within a cross-section of the femur using QCT scans. Through their cross-sectional analysis, they were able to link certain low values of vBMD and Ct.Th to higher fracture risks in the associated area. The present work was not able to correlate Ct.Th and vBMD values to specific areas of increased fracture risk within the radius since the radii have not yet been subjected to dynamic loading tests; however, similar creation of custom

regions of interest were used and Ct.Th and vBMD variation was instead correlated to the clinical standard of aBMD. The current study ultimately found that significant variance in Ct.Th was seen between VOIs and between anatomical ROIs, which implies there could be differing fracture risk around the cortex. A similar study was completed by Long and colleagues (2015) in which they investigated the possibility of using cortical bone thickness estimated from a DXA scan as a predictor of hip fracture risk using the femoral neck. They noted that the Ct.Th is not uniform along either the longitudinal or circumferential direction; thus, they investigated both aBMD and cortical bone thickness within three sites of the femur: narrowest femoral neck, intertrochanter, and femoral shaft. By measuring the cortical thickness inferiorly, superiorly, medially, and laterally using QCT, they were able to deduce a correlation with cortical thickness and aBMD at those sites, with the ultimate goal of finding an alternative way of predicting hip fractures. Current work identified both a positive relationship between Ct.Th and aBMD in both sexes and a positive relationship between aBMD and vBMD, but only in females. Specifically, aBMD and Ct.Th were strongly positively correlated for both sexes ($p<0.0001$) which leads one to believe that Ct.Th is a good predictor of fracture risk. With this in mind, there is still variation left unexplained by Ct.Th, and it has been shown that fracture risk increases independently of T-score and aBMD changes (Bolotin, 2007). Considering aBMD is measuring similar parameters of bone as Ct.Th and vBMD, it is expected that there would be relationships between them; however, there was no correlation between aBMD and vBMD in males. This is likely due to a small sample size and the fact that vBMD is measuring a volume and could be capturing significantly more variation than aBMD is able to. Despite using the femur, Long and colleagues' (2015) study validates the idea that there is significant and important variation within a cross-section that needs to be investigated in other elements, such as the radius, as it might

have biological significance. The present work expands upon these ideals concerning cross-sectional variation and shows there is significant variation present within a cross-section of the radius both within a single ROI (anterior, posterior, medial, lateral) and at one ROI between VOIs (30%, 50%).

A study by Lai et al. (2004) performed a similar cross-sectional analysis in the tibia where they used pQCT to measure Ct.Th and vBMD in order to understand the regional adaptation. It is important to note that their study only utilized females, while the current study consisted of both males and females. They defined anterior, posterior, medial, and lateral regions as their anatomical locations for the cross-sectional analysis and found posterior vBMD was significantly higher than at the anterior cortex, but no differences were seen between the medial and lateral cortices. In terms of Ct.Th, they found the anterior cortical wall showed the greatest thickness compared to the other three regions (Lai et al., 2005). The current work utilized a non-weight bearing bone (radius) when compared to the tibia, but used similar cross-sectional analyses methods. Ultimately, it was found that the medial crest in the radius had the greatest cortical thickness, with it being significantly greater than the lateral ROI in both sexes, and additionally at the anterior and posterior ROIs in males ($p<0.05$). For vBMD, there were no significant differences in vBMD between anatomical regions (ROIs) within a single VOI ($p>0.05$). Therefore, the same trend found in cross-sectional functional adaptation compensation in bone strength by Lai et al. was not observed in the current study. The current study also compared Ct.Th and vBMD at a single ROI between VOIs, while Lai et al. did not. This provided additional information concerning cross-sectional variation. vBMD differences were identified at only the medial ROI ($p<0.01$), with the 30% VOI (males: 1109 ± 29, females: 1095 ± 38) typically larger than the 50% (males: 1089 ± 28, females: 1078 ± 42). Ct.Th differences

were evident between VOIs at all ROIs for females, and at all but the lateral ROI for males ($p<0.01$), with the 50% VOI (males: 1.2 ± 0.15, females: 0.93 ± 0.17) typically larger than the 30% (males: 1.0 ± 0.11, females: 0.77 ± 0.11) for Ct.Th. This inverse relationship is the same pattern observed with total Ct.Th and vBMD earlier in the current study, and it is consistent with the functional compensation found in the tibia by Hunter et al (2017).

Limitations in this study include sample size. A larger sample size could help further characterize the variation in Ct.Th and vBMD. Additionally, the two volumes of interest (VOIs) used in this study (30%, 50%) were utilized in order to remain consistent with related studies; however, it is likely that additional variation along the radius was not accounted for by only selecting two sites. This is particularly likely since the 4% site is clinically the most commonly fractured site. The four anatomical regions (anterior, posterior, medial, lateral) used for the custom regions of interest only encompassed those locations around the cortex, and it is possible that a portion of the variation in both Ct.Th and vBMD was missed. Lastly, the SkyScan protocol used to define the custom regions of interest was standardized; however, no formal quantifications of measurement error (inter-observer or intra-observer) were quantified for the cortex specific analyses of this study, although the process was standardized by a single person. The overall method of QCT scan acquisition and SkyScan data collection has been extensively validated on cortical bone in the radius.

In summary, multiple levels of variation can help approximate and explain how bones respond differently to fracture with load. Understanding all of these different levels of variation is important in fracture risk assessment and could potentially improve clinical fracture assessment techniques. Additionally, while multiple studies have produced differing results concerning sex and age differences in radial vBMD and Ct.Th, this study found the most

prominent differences with sex. Differences in Ct.Th and vBMD were seen with sex; however, there were no significant declines seen with age due to having an elderly sample as previously noted. Ultimately, it is important to further investigate variation in radial vBMD and Ct.Th, since it is unclear how variation plays out at different subject levels. Considering the commonality of forearm fractures in elderly individuals, these results demonstrate the need for additional studies to further investigate the impact of variance in Ct.Th and vBMD for radius fracture risk. Future work will help determine if current clinical methods of measuring bone quality (aBMD) are able to adequately capture relevant variation, and if not, perhaps suggest new methods of assessment.

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